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Partial flooding enhances aeration in adventitious roots of black willow (*Salix nigra*) cuttings

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Summary

Black willow (*Salix nigra*) cuttings are used for streambank stabilization where they are subjected to a range of soil moisture conditions including flooding. Flooding has been shown to adversely impact cutting performance, and improved understanding of natural adaptations to flooding might suggest handling and planting techniques to enhance success. However, data assessing the root aeration in adventitious roots that are developed on cuttings of woody species are scant. In addition, it appears that no data are available regarding aeration of the root system under partially flooded conditions. This experiment was designed to examine the effects of continuous flooding (CF) and partial flooding (PF) on aerenchyma formation and radial oxygen loss (ROL) in black willow cuttings. Photosynthetic and growth responses to these conditions were also investigated. Under laboratory condition, replicated potted cuttings were subjected to three treatments: no flooding (control, C), CF, and PF. Water was maintained above the soil surface in CF and at 10 cm depth in PF. Results indicated that after the 28-d treatments, root porosity ranged between 28.6% and 33.0% for the CF and C plants but was greater for the PF plants (39.2% for the drained and 37.2% for the flooded portions). A similar response pattern was found for ROL. In addition, CF treatment led to decreases in final root biomass and root/shoot ratio. Neither CF nor PF had any detectable adverse effects on plant gas exchange or photosystem II functioning. Our results indicated that *S. nigra* cuttings exhibited

Abbreviations: C, control; CF, continuous flooding; CH_4, methane; Chl, chlorophyll content; Eh, oxidation–reduction potential; F_0, dark fluorescence yield; F_m, maximal fluorescence; F_s, steady-state fluorescence yield; F_{sm}, maximal fluorescence under steady-state conditions; F_v, variable fluorescence; F_v/F_m, efficiency of excitation capture of open photosystem II; g_s, stomatal conductance; PF, partial flooding; PFd, drained zone in partially flooded treatment; PFf, flooded zone in partially flooded treatment; P_n, net photosynthesis; POR, root porosity; PS II, photosystem II; ROL, radial oxygen loss; Rubisco, ribulose 1, 5-biosphosphate carboxylase/oxygenase; SE, standard error; *Y*, yield of energy conversion

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avoidance mechanisms in response to flooding, especially the partially flooded condition which is the most common occurrence in riparian systems.

Introduction

In wetlands, soil flooding initiates a chain of reactions leading to reduced soil oxidation–reduction potential (Eh). Flood-tolerant plants use several strategies to cope with low soil Eh conditions. Aerenchyma development has been considered as a mechanism critical to plant's ability to cope with anaerobiosis. This gas transport system allows plants to transport the atmospheric O_2 to the underground organs to maintain aerobic respiration and to oxidize various reducing compounds in the rhizosphere (Pezeshki, 2001). In some species, poor aeration increases aerenchyma formation and hence porosity (Burdick, 1989; Laan et al., 1989; Armstrong et al., 1994). Aerenchyma forms in roots either lysigenously by cell separation and collapse or schizogenously by cell separation without collapse (Armstrong et al., 1991).

Rhizosphere oxygenation by radial oxygen loss (ROL) from roots is also of great importance for wetland plants to overcome anaerobic conditions. High correlation was found between ROL and soil Eh intensity. It was reported that soil redox potential of −250 mV resulted in enhancement of ROL as much as 3-fold in *Taxodium distichum* compared with those under well-aerated condition (Kludze et al., 1994b). The radial diffusion of O_2 can immobilize or detoxify potential soil toxins including acetic and butyric acids produced by microbial metabolism and soil-reducing compounds (Armstrong, 1979; Mckee et al., 1988; Kludze et al., 1994b). ROL from some plants has been shown to increase soil Eh, which enables those plants to survive in an otherwise anoxic condition (Tessnow and Baynes, 1978). It also supports aerobic nitrifying or nitrogen fixing bacteria in the rhizosphere (Hoffmann, 1990; Ueckert et al., 1990). The potential for nitrification in the rhizosphere is a major consideration underlying the current use of wetlands for the purification in both natural and artificial effluents (Reddy et al., 1989; Armstrong et al., 1994). In addition, ROL from roots to rhizosphere inhibits methanogenesis, promotes methane (CH_4) oxidations and thus reduces potential efflux of CH_4 from the plants. Wetlands contribute 40–50% of total emission of CH_4 to the atmosphere, which accounts for 7–9% of global warming (Armstrong and Armstrong, 2001).

Numerous studies have been conducted to investigate the effects of flooding on root aerenchyma formation and ROL for plants developed from seeds. However, little is known about these processes in adventitious roots that are developed on cuttings of woody species. Jackson and Attwood (1996) evaluated aerenchyma formation of *Salix viminalis* cuttings waterlogged for 4 weeks and noted that aerenchyma in the upper roots (100 mm) was enhanced by flooding. But it appears that no data are available regarding aeration of the root system under partially flooded conditions. This experiment was designed to quantify the effects of continuous flooding (CF) and partial flooding (PF) on root aerenchyma formation and ROL in black willow (*S. nigra*) cuttings under laboratory condition. Black willow is commonly found in floodplains and bottomland hardwood forests of the southeastern United States (Mitsch and Gosselink, 1993). Cuttings of this species are extensively used as planting material for soil stabilization, erosion control, and habitat rehabilitation along highly eroded streambanks (Schaff et al., 2003). Black willow is subjected to dynamic hydrologic conditions in riparian systems. Depending on the slope and depth to base flow, it may be exposed to continuously flooded or partially flooded conditions. Herein we use the expression "partially flooded" to refer to sites where the water table elevation is above much of the root zone. We expected that black willow would be under strong selective pressure to adjust morphologically and physiologically to both continuously flooded and partially flooded conditions in the riparian systems. We hypothesized that: (1) CF would enhance both root porosity (POR) and ROL; (2) roots located in the flooded zone of the PF treatment would have similar aerenchyma formation to those under continuously flooded condition, while those in drained zone should have similar porosity to those in well-aerated condition (i.e., under partially flooded condition, roots located in drained zone would be likely to have less aerenchyma tissue developed than those grown in flooded zone). PF would also stimulate ROL from roots (both zones combined); (3) due to these morphological responses, minimal disruption of photosynthetic and growth parameters would be shown under CF and PF, which would be indicated by no reduction in net photosynthesis (P_n), stomatal conductance (g_s), chlorophyll content (Chl), height growth, and biomass; and (4) no change would be detected in chlorophyll fluorescence parameters such as dark fluorescence yield (F_0), efficiency of excitation capture of open photosystem II (PS II; F_v/F_m) in dark-adapted leaves and yield of energy conversion (Y), which suggests the resilience of PS II to flooding.

Materials and methods

Plant materials

Black willow (*Salix nigra* Marshall) cuttings were collected from a localized population on the Loosahatchie River in western Tennessee, USA on June 1, 2004. Each cutting was 0.5 cm in diameter at the base and 25 cm in length. All existing branches were removed from each cutting to conform to common planting practices.

Experimental procedures

Cuttings were planted on June 2 in a laboratory. Pots 25 cm deep and 5 cm in diameter were constructed of PVC pipe and filled with two parts sand and one part soil (v/v) up to 20 cm. Caps were glued to the bottom of each pot, and holes were drilled in the sides of the pots to allow control of soil moisture regimes. A single cutting was planted in each pot with 10 cm below the soil surface and 15 cm above the soil surface. In order to allow for more uniformity, those performing poorly were excluded. Environmental conditions were day/night temperature of 25/20 °C, a 16 h photoperiod, and photosynthetic photon flux density of 1000 μmol m^{-2} s^{-1} at the top of the plants. Plants were maintained under well-watered and well-drained conditions prior to the treatment initiation. Cuttings were fertilized with 50 mL of Peters Fertilizer 20–20–20 mixed with tap water at 1.25 g L^{-1} on June 23, 27, July 2, 9, 13, 16, 19, 22, and 26. The mean height of all cuttings was 19.6 cm ($\pm$0.8) when the treatments were initiated on July 28. Eight plants were harvested and the initial biomass was recorded. The mean shoot and root biomass (dry weight) were 0.48 g ($\pm$0.03) and 0.35 g ($\pm$0.06) before the treatment initiation. Plants grew under each treatment for 28 d.

Three treatments were used to test the responses of willow cuttings to soil moisture regimes. The treatments represented a relatively wide range of soil moisture that could be expected in the field during the growing seasons. These treatments were: (1) control (C), cuttings were watered daily and allowed to drain freely; (2) CF, cuttings were flooded to 5 cm above the soil surface throughout the experiment; and (3) PF, a second hole was drilled at 10 cm from the bottom of each pot to allow the water level to be maintained at 10 cm below the soil surface for the duration of the study. Each treatment consisted of a total of 30 replicate cuttings.

Soil measurements

Soil redox potential (Eh) was monitored using platinum-tipped electrodes, a Model 250 A ORION redox meter and a calomel reference electrode (Thermo Orion, Beverly, MA, USA) as described in detail elsewhere (Patrick and DeLaune, 1977) at 10 cm below the soil surface for C and CF groups. There were two measurements for PF treatment, at 5 cm below the soil surface for the upper drained portion and at 15 cm below the soil surface for the lower flooded portion. Measurements were replicated six times per measurement day on days 0, 7, 14, 21, and 28 for C, CF as well as both the upper drained and lower flooded zones of PF. The Eh values were corrected according to Patrick and DeLaune (1977). An Eh value of+350 mV represents the approximate level at which O_2 begins to disappear from the soil. Well-aerated conditions are represented by +400 to +700 mV, while reduced condition may produce Eh values as low as –300 mV (DeLaune and Pezeshki, 1991).

Root porosity

POR, a measurement of the percentage air space within a root, was also measured on days 0, 7, 14, 21, and 28. Four plants for each of the three treatments were harvested at random. Root systems from the drained portion and flooded portion of the same plant under PF condition were separated. Root samples weighing 0.4–0.6 g were cut from an area of new growth. Measurements were taken using 50 mL pycnometer as described by Jensen et al. (1969), Kludze et al. (1993), Kludze et al. (1994a), and Kim et al. (1999). The pycnometer was first filled with water and weighed. The roots were weighed and placed in the water-filled pycnometer, and then weighed. Next, the roots were extracted and ground to a paste with mortar and pestle. The ground roots were placed in water-filled pycnometer and weighed. POR was determined by

$$\mathrm{POR} = [(p_{\mathrm{gr}} - p_{\mathrm{r}})/(r + p - p_{\mathrm{r}})]100,$$

where POR is the root air-space or porosity (%), p_{gr} the mass of pycnometer with water and ground roots (g), p_r the mass of pycnometer with water and roots (g), r the mass of roots (g), p the mass of pycnometer with water (g).

Radial oxygen loss

A colorimetric technique involving the use of titanium (III) citrate solution was used to assay O_2 released from black willow roots at the conclusion of the experiment (four plants per treatment) as described by Kludze et al. (1993). The Ti^{3+} citrate was prepared under N_2 atmosphere according to Zehnder and Wuhrman (1976). Three hundred milliliters of deoxygenated deionized water was added to 17.65 g of sodium citrate to give 0.2 M sodium citrate solution. Thirty milliliters of 15% titanium chloride solution (Aldrich chemical company, Inc, Milwaukee, WI, USA) was then added to the sodium citrate solution. The initial pH of the Ti^{3+} citrate solution was about 2 and was adjusted to 5.6 by adding saturated sodium carbonate.

One hundred milliliters of deoxygenated deionized water was poured into 250 mL bottles previously evacuated of air, and N_2 was bubbled through the water for 20 min to remove dissolved O_2. Roots along with 15 cm cuttings were gently washed of any foreign matter with deionized water. Parafilm was used to coat 5 cm of the cuttings (right above the 15 cm line) and then inserted into the bottles with roots completely immersed in the water (one plant per bottle). The parafilm coating was done to prevent shoot exposure to paraffin oil layer and to eliminate any possible O_2 leakage from shoot lenticels or wound in the estimation of ROL (Kludze et al., 1993). A 5 mL aliquot of Ti^{3+} citrate was injected into each bottle with a plastic syringe, followed by layering the solution surface with 2 cm thick of paraffin oil to hinder atmospheric O_2 contamination. The control treatment bottle was prepared in an identical fashion, but with no plant material. After 6 h, the bottles were gently shaken and the solution was taken with a syringe through rubber tubing that had been introduced into the solution alongside the roots. Solution lost by transpiration was replaced through the rubber tubing.

Absorbance of the partly oxidized Ti^{3+} citrate solution was measured at 527 nm on a Spectronic 20 spectrophotometer (Milton Roy Company, Rochester, NY, USA). Released O_2 was determined by extrapolation of the measured absorbance to a standard curve obtained from a dilution series of the Ti^{3+} citrate solution being used

$$\mathrm{ROL} = c(y - z),$$

where ROL is the radial oxygen loss (μmol O_2 plant^{-1} h^{-1}), c the initial volume of Ti citrate added to each flask (L), y the concentration of Ti^{3+} citrate solution of control (without plants) (μmol Ti^{3+} L^{-1}), z the concentration of Ti^{3+} citrate solution after 6 h with plants (μmol Ti^{3+} L^{-1}).

Plant photosynthetic responses

Measurements of plant photosynthetic responses were made on the same day as listed for soil Eh. All photosynthetic measurements were conducted between 9:00 a.m. and 12:00 noon. Measurements of photosynthesis (P_n) and g_s were obtained on five plants randomly chosen per treatment, one measurement per plant, on each sampling day. Measurements were conducted on the upper third fully developed leaf from the tallest shoot apex using a portable gas exchange analyzer (CIRAS 1, PP Systems, Haverville, MA, USA). Immediately after the measurement of gas exchange, leaf Chl was recorded on the same leaves using a CCM-200 Chl meter (Opti-sciences, Tyngsboro, MA, USA).

Leaf chlorophyll fluorescence was measured with the Model OS-100 Modulated Fluorometer (Opti-Sciences, Tyngsboro, MA, USA) on the same leaves used for P_n, g_s, and Chl. Three records were made as follows: (1) F_0, (2) F_v/F_m: $F_v/F_m = (F_m - F_0)/F_m$, where F_m is the maximal fluorescence obtained with a 0.8 s saturation flash, F_v is the variable fluorescence and (3) yield of energy conversion (Y): $Y = (F_{sm} - F_s)/F_{sm}$, where F_{sm} is the maximal fluorescence obtained with a 0.8 s saturation flash under steady-state conditions, and F_s is the steady-state fluorescence yield under ambient irradiance.

Plant growth

Plant height growth was determined by calculating the differences between the height measured before the initiation of the treatments and at the conclusion of the experiment (day 28) for eight plants per treatment. Then five plants were separated into aboveground and belowground portions. The aboveground biomass was further divided into shoots (branches and leaves) and aboveground cutting. Belowground biomass was separated into roots and belowground cutting. All shoots and roots were dried at 70 °C to a constant weight and final dry weights were recorded. Root/shoot ratio was calculated as the ratio of dry root weight to dry shoot weight.

Data analyses

The experiment followed a completely randomized design. Two-way MANOVA (SPSS 11.5) with three levels of flooding and five levels of sampling date was used to test the differences in means for photosynthetic responses including P_n, g_s, and Chl as well as for PS II parameters including F_0, F_v/F_m, and *Y*. Two-way ANOVA (SPSS 11.5) with flooding and sampling date was used to test the differences in means of POR. One-way ANOVA (SPSS 11.5) was used to test the differences in means of ROL, height growth, and biomass between flooding treatments. The Tukey procedure was used to examine all pairwise group differences. Differences were considered significant at $p<0.05$.

Results

Soil measurements

Soils in all groups were aerated on day 0 before the initiation of treatment. Soil Eh in C remained above +493 mV for the duration of the experiment, indicating oxic condition in this treatment. But soil was reduced in CF after the treatment initiation and the level of soil Eh in this treatment remained in the mildly reduced range (−4 to −104 mV) for the duration of the study. In PF treatment, soil was oxic in the upper drained portion (above +455 mV) but mildly reduced in the lower flooded zone (+ 9 to+118 mV) on days 7, 14, 21, and 28 (Fig. 1).

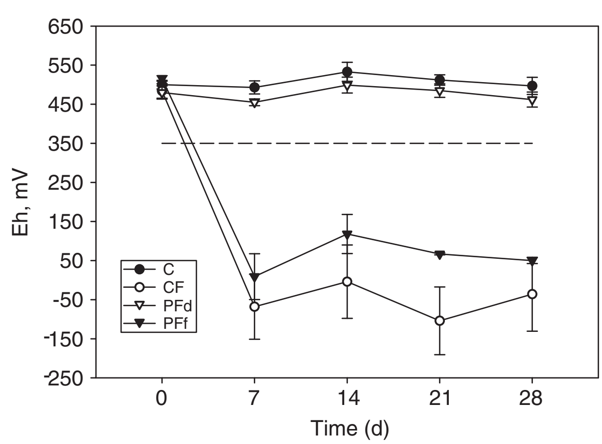

Figure 1. Time-course changes of soil redox potential (Eh) recorded for control (C), continuous flooding (CF), and partial flooding (PFd for drained zone and PFf for flooded zone) treatments. The dashed line at +350 mV represents the approximate level at which O_2 begins to disappear from the soil. Each value is the mean for six replications ± standard error (SE).

Root porosity and radial oxygen loss

No interaction between flooding and sampling date was detected for POR ($F_{(12,60)} = 1.21$, $p = 0.299$). The main effect of flooding was shown to be significant ($F_{(3,60)} = 3.99$, $p = 0.012$). After the 28-d treatments, POR ranged between 28.6% and 33.0% for the CF and C plants but was enhanced in PF plants (39.2% for the roots located in the drained and 37.2% for those in the flooded portions, Fig. 2).

The results of ROL showed a similar pattern as found for POR, with PF cuttings characterized by the greatest value ($F_{(2,9)} = 5.293$, $p = 0.030$, Fig. 3). Mean ROL in PF group was 1.23 and 1.59 times that of C and CF plants, respectively.

Photosynthetic responses

The MANOVA results indicated that there was no significant interaction between flooding and day of measurement for photosynthetic responses ($F_{(24, 168.82)} = 1.033$, $p = 0.428$). The simple effect of flooding effect, however, was significant ($F_{(6, 116.00)} = 2.666$, $p = 0.018$). Nevertheless, the results of the pair-wise comparisons (both multivariate and univariate tests) between each pair of flooding indicated that there were no differences in P_n, g_s or Chl between the three treatments (Table 1).

Likewise, there was no significant interaction between flooding and day of measurement for the three measured PS II parameters ($F_{(24, 168.82)} =$ 0.865, $p = 0.649$). The effect of flooding on these

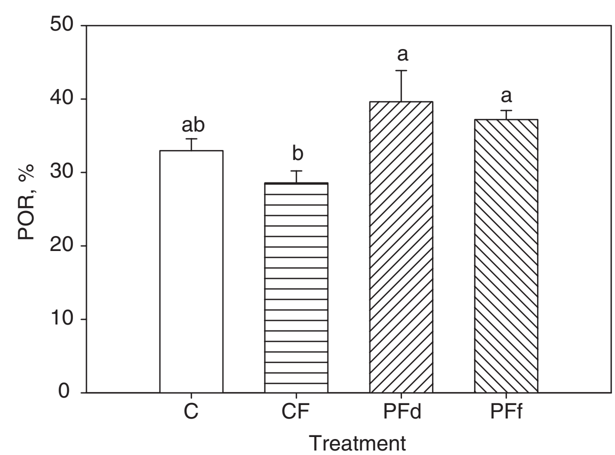

Figure 2. Root porosity (POR) for black willow cuttings in control (C), continuous flooding (CF), and partial flooding (PFd for drained and PFf for flooded zone). Each value is the mean for 20 measurements (four replications × five days) ± SE. Significant differences are shown across treatments using different letters.

parameters was not detectable either ($F_{(6, 116.00)} = 1.604$, $p = 0.152$).

Plant growth

Neither height growth nor final shoot biomass was significantly different across treatments ($F_{(2, 21)} = 0.399$, $p = 0.676$ and $F_{(2, 12)} = 3.042$, $p = 0.085$, respectively, Table 2). The final root biomass, however, was lower in CF as compared with C and PF ($F_{(2, 12)} = 9.180$, $p = 0.004$, Fig. 4A). Thus the lowest root/shoot ratio of 0.53 was recorded for plants grown under CF ($F_{(2, 12)} = 7.452$, $p = 0.008$, Fig. 4B).

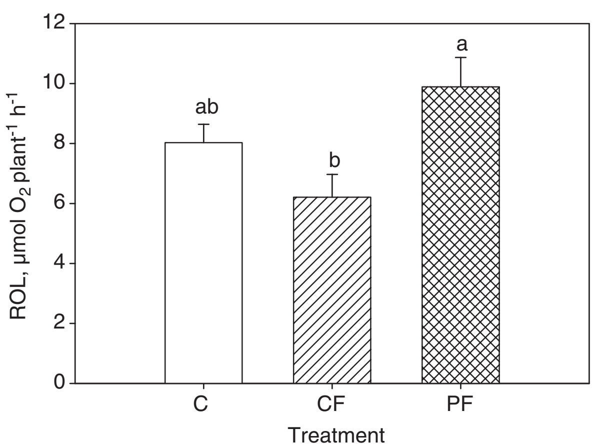

Figure 3. Radial oxygen loss (ROL) for black willow cuttings in control (C), continuous flooding (CF), and partial flooding (PF) at the conclusion of the experiment. Each value is the mean for four measurements ± SE. Significant differences are shown across treatments using different letters.

Discussion

Results from this study indicated that *S. nigra*, like many other wetland species (Smirnoff and Crawford, 1983), is capable of constitutively developing extensive aerenchyma tissue even in drained roots (Fig. 2). In some species, root aerenchyma is further promoted by hypoxia (Justin and Armstrong, 1987; Jackson and Attwood, 1996; Chen et al., 2002; Colmer, 2003) to enhance internal transfer of atmospheric or photosynthetic O_2 between the plant parts above the water and the flooded tissues and to sustain root aerobic respiration and growth (Armstrong, 1979). We did not find the enhancement of gas space in roots of CF cuttings as compared with C as proposed (first hypothesis). The reason might be that the soil reduction was mild (as shown by Eh data, Fig. 1).

As proposed (second hypothesis), average root POR value (39.2%) in the roots located in the drained zone of PF plants was comparable with that recorded for roots of C plants. However, POR for roots located in the flooded zone of PF treatment (37.2%) was found to be even greater than that of

Table 1. Pair-wise comparison (F value, df, and p value) between flooding treatments (control, C; continuous flooding, CF; and partial flooding, PF) for net photosynthesis (P_n), stomatal conductance (g_s), and chlorophyll content (Chl) for black willow cuttings

Pair	Multivariate tests			Univariate tests						
	df	F	p	df	P_n		g_s		Chl	
					F	p	F	p	F	P
C-CF	3,46	1.553	0.214							
C-PF	3,46	2.792	0.051	1, 48	0.015	0.902	1.584	0.214	1.490	0.228
CF-PF	3,46	3.337	0.027	1, 48	0.472	0.495	0.563	0.457	3.052	0.087

The significance level used was $p < 0.05$.

Table 2. Height growth and final shoot biomass for black willow cuttings in control (C), continuous flooding (CF), and partial flooding (PF)

Variable	C	CF	PF
Height growth (cm)	9.91 (±2.30)	7.61 (±1.59)	8.84 (±1.47)
Shoot biomass (g)	1.44 (±0.04)	1.36 (±0.13)	1.66 (±0.07)

Each value is the mean for five replications ± SE.

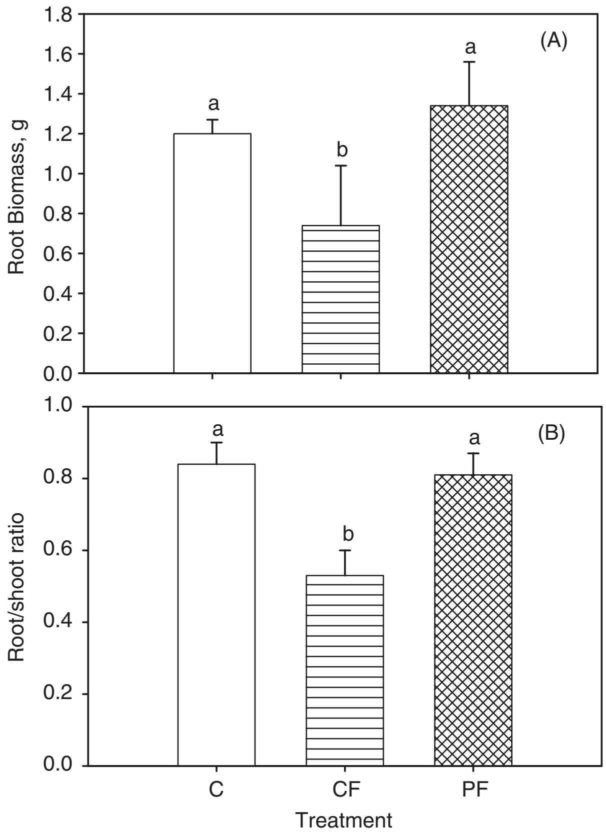

Figure 4. Final root biomass (A) and root/shoot ratio (B) for black willow cuttings in control (C), continuous flooding (CF), and partial flooding (PF). Each value is the mean for five replications $\pm$SE. Significant differences are shown across treatments using different letters.

roots in plants grown under CF treatment (Fig. 2). This was interesting because the soil was aerated in the top drained zone of PF treatment and the soil Eh was slightly milder in the flooded zone of PF treatment than recorded for CF treatment (Fig. 1). More aerenchyma formation could be expected for roots grown under CF treatment with more reduced soil Eh condition than soils in PF treatment since a positive correlation has been established between the intensity of soil reduction and aerenchyma formation in several species (Kludze et al., 1993; Kludze and DeLaune, 1994; Pezeshki and Anderson, 1997). It is known that there are several kinds of signals by which stressed roots influence shoots through the transpiration stream (Jackson, 1993, 2002). We assumed that flooded roots might have conveyed some signal to the roots in the drained zone under PF conditions, which may have caused the roots in both zones to develop more aerenchyma than roots of CF plants. There appears to be no published work comparing root POR in root systems that are partially drained and partially flooded. Therefore, this suggested signaling system merits further investigation.

Increase of ROL is another important adaptive response to waterlogging in wetland plants. A positive relationship has been established between the intensity of soil reduction and both aerenchyma formation and ROL (Kludze et al., 1993; Kludze and DeLaune, 1995). However, our data did not support the hypotheses (first and second) that flooding would lead to elevated ROL. We did observe that PF cuttings had slightly higher ROL. The lack of evidence for enhancement of ROL in CF plants could be attributed to the mildly reduced soil conditions. In addition, the present study did show that aerenchyma formation and ROL were both greatest in the PF treatment (Fig. 3). ROL was tested for individual plants with the unit being $\mu mol\ O_2\ plant^{-1}\ h^{-1}$. Therefore, O_2 releases from the roots in the drained and flooded zones of PF plants could not be determined separately. Again, we speculated that there was a signaling system between the roots from these two zones under PF treatment, which enhanced ROL in this group.

Reduced plant photosynthetic capacity in response to soil flooding is reported for many species (Pezeshki, 2001 and the references therein). The reduction in P_n of woody plants subjected to flooding can be attributed to the closing of stomata (Kozlowski, 1997) and some non-stomatal factors (Pezeshki, 2001). For example, ethylene has been implicated in the photosynthetic decline in *Glycine max* (Taylor and Gunderson, 1988). Nevertheless, the role ethylene plays in such a decline requires additional research. Reduction in P_n could also be the result of reduced ribulose 1, 5-biosphosphate carboxylase/oxygenase (Rubisco) activity (Pezeshki, 1994). Pezeshki et al. (1998) reported that P_n and g_s in *S. nigra* cuttings were significantly lower in partially and continuously flooded treatments as compared to control. However, our results supported our hypothesis that no reductions in P_n, g_s or Chl were expected in either CF or PF plants (third hypothesis, Table 1). The reasons might be that the soil Eh conditions under both CF and PF were milder in the present study as compared to the previous one. In addition, root aerenchyma formation under CF and PF as well as the ability of PF cuttings to release more O_2 than C cuttings help this species maintain the normal photosynthetic rates under flooded conditions in the present study.

Chlorophyll fluorescence can reveal the responses of a plant to environmental stresses and the extent to which those stresses have damaged the photosynthetic apparatus (Fracheboud et al., 1999; Mauchamp and Méthy, 2004). Chlorophyll fluorescence parameters such as F_0, F_v/F_m, and Y

are thought to be good indicators of the effects of environmental stresses on photosynthesis (Ball et al., 1994; Maxwell and Johnson, 2000) and could be used as a tool to investigate the down-regulation of PS II photochemistry parallel to that of photosynthesis. Throughout the experiment, we did not find any differences in F_0, F_v/F_m, or Y among treatments. This indicated that there was no damage to photochemical reactions, which supported our gas exchange results (Table 1) and our hypothesis (fourth hypothesis).

Results of final root biomass measurements indicated that CF cuttings accumulated lower final root biomass than other treatments, which was responsible for the lowest value of root/shoot ratio noted in this treatment (Fig. 4A and B). Even though we did not expect the root growth to be adversely affected by flooding owing to the morphological adaptation (third hypothesis), our results confirmed the sensitivity of roots to CF. It is known that stem lenticels of many woody species, such as *Mangifera indica*, immediately above the submergence line provide the major entry point for O_2 to the shoot–root gas–space system (Schaffer, 1998). Therefore, under CF, there was at least an extra 5 cm of internal diffusion path resistance and shoot cortical O_2 consumption (at least during the night) between the atmosphere and the closest root–shoot junction. This may have contributed to the poorer performance in CF cuttings. Another possible reason is that the cortical porosity of the shoot would be relatively low. Consequently, the roots of the CF plants would have experienced significantly more O_2 stress than PF or C plants (particularly at night) and this could have been exacerbated by the relatively high temperatures used in the experiment. It is also known that stimulation of aerenchyma is due to increased ethylene synthesis, attributed to enhanced production of 1-aminocyclopropane-1-carboxylic acid in roots that are partially deficient in O_2 (Jackson, 1982). Therefore, if internal O_2 concentration in roots is too low (anoxic condition), ethylene fails to stimulate aerenchyma and this might have also been responsible for the slightly lower POR in CF plants (Fig. 2).

Decreased shoot biomass and height growth in response to soil flooding have also been reported for some species (Pezeshki and DeLaune, 1998; Brown and Pezeshki, 2000); however, no reductions in final shoot biomass or height growth (Table 2) were found in the present study, which supported our hypothesis (third hypothesis). It is important to note that the ability to develop aerenchyma tissue and release O_2 to the rhizosphere may be partially responsible for allowing this species to maintain the normal shoot growth under CF and PF treatments.

In conclusion, average POR was higher for both drained and flooded root portions of PF plants than that in CF and C plants. Likewise, the greatest amount of ROL was detected in PF cuttings. The potential signaling system between roots in two zones (drained vs. flooded) under PF treatment needs further confirmation. As hypothesized, neither CF nor PF treatment had any detectable adverse effects on plant gas exchange or PS II functioning, which corresponded to the results of final shoot biomass and height growth. CF treatment changed the pattern of biomass allocation and led to decreases in root/shoot ratio. Our results indicated that *S. nigra* cuttings exhibited a number of avoidance mechanisms in response to flooding, especially PF, that included enhanced root aerenchyma tissue, increased O_2 release into the rhizosphere, unaffected photosynthetic activity, final shoot biomass, and height growth. Findings suggest that cuttings conditioned by growth in a prolonged, partially flooded state may be able to withstand flooding better due to these strategies.

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